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Enhancement of the Immune Response to the *Yersinia pestis* F1-V Candidate Vaccine by CpG Oligonucleotides

Kei Amemiya

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Why do we want to evaluate CpG oligonucleotides?

The current candidate plague vaccine is F1-V, which can confer protection, but it requires multiple vaccinations to attain protection.

Purpose of this study:

1. Examine the murine immune response to a single low dose of rF1-V.

2. Determine the effect of CpG ODNs on the immune response to rF1-V.

F1-V is a recombinant fusion protein consisting of two subunits: a capsular protein designated fraction 1 or F1 and a V antigen (or LcrV).

1. F1 (150aa, 15.7 kDa)
   a. The structural gene or *caf*1 is on a 100 kb plasmid (pFra), one of three plasmids associated with *Yersinia pestis*.
   b. Expression of F1 is dependent on temperature (37 °C).
   c. Appears to have antiphagocytic activity.
   d. F1- strains are still pathogenic.

2. V - antigen (326aa, 37.2 kDa)
   a. The structural gene is on a 70 kb plasmid common to all *Yersinia* species.
   b. Expression of V is dependent on temperature and low Ca^{2+}.
   c. Biological properties:
      i. Required for the expression and translocation of specific Yops as part of the type III secretion system.
      ii. Suppresses host expression of TNF-α and IFN-γ.
      iii. Inhibits neutrophil chemotaxis.

CpG Oligonucleotides (ODN)s: Structure-Function

1. **CpG-A ODN (D-type)**
   a. Effective in activating NK cells and inducing IFN-α production from plasmacytoid DC precursors.
   b. Phosphodiester linkages flanking a central CpG motif and may have phosphorothioate(PS) linked bases on the ends.
   c. Poly G motif at their 5' and/ or 3' ends.

   5'-GGTGCATCGATGCAGGGGGG-3'

2. **CpG-B ODN (K-type)**
   a. Enhanced B cell stimulatory properties and reduced NK stimulation.
   b. Thymidine 5' to the CpG and TpT or ApT in the 3' position.
   c. May have multiple CpG dinucleotides.
   d. PS linked bases throughout the ODN.
   e. No poly G motifs.

   5'-TCCATGACGTTCCTGACGTT-3'

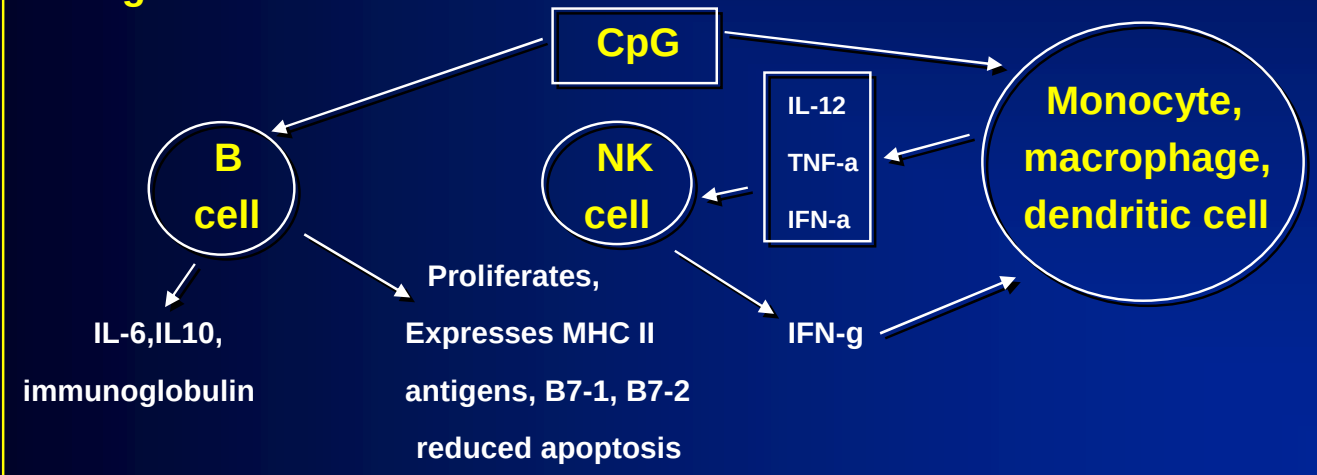
From: Krieg Annu.Rev.Immunol. 2002. vol 20,p709; Klinman etal.,Microbes and Infect. 2002. vol 4,p897.

Activity of CpG Oligonucleotides

Structure

5'-TCCATGACGTTCCTGACGTT-3'	Active
5'-TCCAGGACTTCTCTCAGGTT-3'	Nonactive

Biological activities



- From: A.M. Krieg et al., 1998, Trends in Microbiol. vol.6, p23.

Protocol

1. Varying amounts of F1-V (0.1-1.0 *u*g) are used with and without different amounts of CpG ODNs (5-25 *u*g) with a constant amount of Alhydrogel.

2. BALB/c mice (6-8 wks old) are vaccinated subcutaneously with the mixture.

3. The mice are challenged parenterally 30 days later with 5 X 10>7 CFU of *Y. pestis* CO92.

4. Serum and spleens (if needed) are collected from a set of mice before challenge and from surviving mice 21 days later.

CpG ODNs Evaluated

- Set no. 1:
- 10105 5'-TCGTCGTTTTGTCGTTTTTTTCGA-3' (24 mer)
- 4008 5'-TGCTGCTTTTGTGCTTTTTTTGCA-3' (24 mer)
- Set no. 2:
- 1826 5'-TCCATGACGTTCCTGACGTT-3' (20 mer)
- 1982 5'-TCCAGGACTTCTCTCAGGTT-3' (20 mer)
-

The Immune Response to F1-V is Enhanced by CpG ODN 5470.

Group	F1-V (*u*g)	CpG (*u*g)	Titer IgG	IgM	IgG1	IgG2a	Ratio IgG2a/IgG1
1.	0.1	0	69,500	50.0	420,000	1012	0.0024
2.	0.5	0	120,000	50.0	520,000	900	0.0017
3.	1.0	0	144,000	50.0	520,000	312	0.0006
4.	0.1	5	288,000	87.5	960,000	21,400	0.0223
5.	0.1	10	112,400	50.0	853,300	91,730	0.1075
6.	0.1	25	150,000	50.0	243,200	80,850	0.3324
7.	0.5	5	1,200,000	62.5	3,520,000	9,600	0.0027
8.	0.5	10	190,000	50.0	1,280,000	15,400	0.0120
9.	0.5	25	290,000	87.5	3,380,000	64,050	0.0189
10.	1.0	5	1,200,000	62.5	2,880,000	26,000	0.0090
11.	1.0	10	840,000	<50	2,880,000	80,400	0.0279
12.	1.0	25	600,000	50.0	4,800,000	168,400	0.0351
13.	0	5	<50	50.0	250.00	75.0	ND
14.	0	10	<50	50.0	50.0	237.5	ND
15.	0	25	50.0	50.0	137.5	50.0	ND

The Immune Response to F1-V is Slightly Enhanced by NonODN 4004.

Group	F1-V (*u*g)	CpG (*u*g)	Titer				Ratio
			IgG	IgM	IgG1	IgG2a	IgG2a/IgG1
1.	0.1	5	72,000	<50	176,000	425	0.002
2.	0.1	10	104,000	50	336,000	1,030	0.003
3.	0.1	25	20,300	<50	27,000	50	0.002
4.	0.5	5	108,000	50	320,000	2,050	0.006
5.	0.5	10	260,000	62.5	1,460,000	7,200	0.005
6.	0.5	25	32,000	75	240,000	550	0.002
7.	1.0	5	144,000	50	880,000	1,080	0.001
8.	1.0	10	176,000	50	1,040,000	5,330	0.005
9.	1.0	25	168,000	50	960,000	9,700	0.010

The Murine Immune Response to the *Y. pestis* F1-V Vaccine is Enhanced by CpG ODN 1826

Group	F1-V (*u*g)	CpG (*u*g)	Titer				Ratio
			IgG	IgM	IgG1	IgG2a	IgG2a/IgG1
1.	0.1	5	1,200,000	50	1,920,000	80,000	0.042
2.	0.1	10	501,000	75	2,030,000	173,000	0.085
3.	0.1	25	500,000	50	830,000	82,000	0.099
1.	0.5	5	2,920,000	63	1,440,000	212,000	0.147
2.	0.5	10	840,000	88	3,520,000	86,400	0.025
3.	0.5	25	1,280,000	225	2,560,000	232,000	0.906
4.	1.0	5	2,400,000	487	2,560,000	768,000	0.300
5.	1.0	10	1,120,000	63	3,070,000	80,000	0.026
9.	1.0	25	1,920,000	150	7,680,000	322,000	0.042

The Protective Response of F1-V is Modulated by CpG ODN 5470.

Group	Treatment F1-V (*u*g)	Treatment CpG (*u*g)	No. Survivors (After 21 Days)	MST (± SE) (Days)
1.	----	----	0/6	3.5 (0.56)
2.	0.1	----	0/10	4.5 (0.58)
3.	0.5	----	2/10	10.8 (1.81)
4.	1.0	----	5/10	14.3 (2.47)
5.	0.1	5	6/10	14.8 (2.78)
6.	0.1	10	5/10	15.0 (2.14)
7.	0.1	25	2/10	9.3 (2.19)
8.	0.5	5	10/10	21.0 (0.00)
9.	0.5	10	7/10	17.2 (2.29)
10.	0.5	25	1/10	7.4 (1.64)
11.	1.0	5	9/10	19.9 (2.29)
12.	1.0	10	7/10	16.7 (2.57)
13.	1.0	25	8/10	17.8 (2.86)
14.	----	5	0/10	3.4 (0.16)
15.	----	10	0/10	3.6 (0.22)
16.	----	25	0/10	3.6 (0.27)

The Enhancement of the Immune Response to F1-V by CpG ODN 5470 Can be Seen in the Survivors After Challenge.

Group	F1-V (*u*g)	CpG (*u*g)	Titer				Ratio IgG2a/IgG1
			IgG	IgM	IgG1	IgG2a	
3.	0.5	0	10,240,000	600	7,680,000	15,000	0.0019
4.	1.0	0	6,400,000	325	5,760,000	44,000	0.0076
5.	0.1	5	5,120,000	375	3,840,000	340,000	0.0885
6.	0.1	10	7,200,000	300	5,800,000	1,460,000	0.2517
7.	0.1	25	15,360,000	300	6,400,000	640,000	0.1000
8.	0.5	5	8,960,000	150	9,600,000	960,000	0.1000
9.	0.5	10	12,160,000	225	17,280,000	1,280,000	0.0741
10.	0.5	25	10,240,000	200	51,200,000	5,120,00	0.1000
11.	1.0	5	12,800,000	250	89,600,000	1,760,000	0.0196
12.	1.0	10	9,600,000	300	19,200,000	440,000	0.0229
13.	1.0	25	11,520,000	800	14,400,000	1,080,000	0.0750

The NonCpG ODN 4008 Does Not enhance the Protective Response of F1-V

Group	F1-V (*u*g)	CpG (*u*g)	No. Survivors	MST (Days) (±SE)
1.	0.1	5	1/10	9.2 ± 1.57
2.	0.1	10	0/10	6.5 ± 0.34
3.	0.1	25	0/10	5.0 ± 0.26
4.	0.5	5	2/10	11.8 ± 1.59
5.	0.5	10	2/10	10.3 ± 1.80
6.	0.5	25	2/10	10.2 ± 1.85
7.	1.0	5	2/10	10.4 ± 1.83
8.	1.0	10	0/10	9.6 ± 0.65
9.	1.0	25	0/10	8.1 ± 0.65

Conclusion

1. F1-V with alhydrogel induces predominately a Th2-like immune response (IgG1) in our plague mouse model.

2. The murine antibody response to F1-V can be enhanced by CpG ODNs but not by nonCpG ODNs.

3. The antibody response is shifted toward a Th1-like direction (IgG2a) by CpG ODNs.

4. The protective response to low concentrations of F1-V can be enhanced by low concentrations of CpG ODNs.

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